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Journal of Chromatography B, 789 (2003) 101–105
JOURNAL OF CHROMATOGRAPHY B
www.elsevier.com/locate/chromb

Rapid high-performance liquid chromatographic measurement of amisulpride in human plasma: application to manage acute intoxication

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Abstract

Amisulpride, a substituted benzamide derivative, is a second-generation (atypical) antipsychotic and is effective as maintenance therapy in patients with schizophrenia. For toxicological purpose, a rapid RP-HPLC assay was developed for the determination of amisulpride in human plasma. A linear response was observed over the concentration range 100–1000 ng/ml. A good accuracy (≤5%) was achieved for all quality controls, with intra- and inter-day variation coefficients equal or inferior to 4.9%. The lower limit of quantification was 20 ng/ml, without interferences of endogenous components. This rapid method (run time <5 min) was used to monitor eight intoxications involving amisulpride.

Keywords: Poisoning; Amisulpride

1. Introduction

Amisulpride is an orthomethoxy benzamide derivative chemically related to sulpiride. This CNS agent presents a high affinity for dopamine D2 and D3 receptors and demonstrates antischizophrenic and antidysthymic (antidepressant) properties in man [1,2]. This drug is effective as maintenance therapy in patients with chronic schizophrenia and generally a long-term treatment with amisulpride was associated with improvement in quality of life and social functioning [3].

Amisulpride is generally well tolerated and the neurological tolerability profile is superior to that of conventional antipsychotics (haloperidol and flupenthixol) [4,5]. Amisulpride poisoning may induce seizures, QT prolongation and *torsades de pointes* [6]. Consequently, it is essential to use a rapid and specific method for the determination of amisulpride in biological fluids in case of acute intoxication.

The published methods for amisulpride analyses in biological fluids described HPLC assays with fluorescence detection [7–9]. These methods were developed for pharmacokinetic studies especially in clinical investigations with low dosage treatments, and they required sensitivity.

The aim of this study was to establish a RP-HPLC method for the measurement of amisulpride in case of acute poisoning. The quantitation of plasma levels of amisulpride was performed using a simple liquid–liquid extraction procedure in the presence of an

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1570-0232/03/$ – see front matter
doi:10.1016/S1570-0232(03)00045-X

internal standard, viloxazine. It presents several advantages, such as rapidity and selectivity and consequently, it is especially adapted for the management of polyintoxications.

2. Experimental conditions

2.1. Chemicals

Amisulpride was kindly provided by Sanofi-Synthelabo (Le Plessis Robinson, France). The internal standard, viloxazine was obtained from Zeneca Pharma (Cergy, France). All reagents used for the assay were of HPLC or analytical grade. The reagent containing sulfonic pentane acid (Pic B5®*Low UV*) was a premixed product of Waters (Milford, MA, USA). The phosphate buffer (6.2×10^{-2} *M*) was prepared by dissolving 9.08 g of KH_2PO_4 and 11.60 g of K_2HPO_4 in 1000 ml of water. Water was deionized and glass distilled prior to use and human heparinized plasma of healthy volunteers was purchased from Aquitaine Establishment of Blood Transfusion (E.T.S.A, Bordeaux, France).

2.2. HPLC conditions

The chromatographic apparatus (ThermoQuest™, San Jose, CA, USA) was equipped with a constant flow pump M 100, a Model 150 ultraviolet detector and a Datajet®integrator.

The chromatographic separation was performed at room temperature on a Spherisorb® S5 C_8 analytical column (Waters) (4.6×150 mm; 5-μm particle size). The mobile phase consisted of acetonitrile–phosphate buffer (6.2×10^{-2} *M*) (25:75, v/v). To this mixture, 500 μl of diethylamine and a vial of Pic B5 was added for 1 l. In final, the pH of this eluent was adjusted to 6.4 with orthophosphoric acid. This mobile phase was filtered through a 0.45 μm filter and degassed prior to use. The flow rate was maintained at 2 ml/min. The compounds were detected at 280 nm for 5 min. The unknown concentrations of amisulpride were quantified using a linear regression model of response (drug/I.S. peak height ratio) versus amisulpride concentrations.

2.3. Standard solutions

Stock standard solutions of amisulpride and viloxazine (internal standard) were prepared at concentrations of 1 mg/ml in methanol and stored at −20 °C. Daily, the internal standard (I.S.) stock solution was diluted in bidistilled water to yield a 30 μg/ml working solution. A standard solution of amisulpride was prepared from the stock solution by suitable dilution with distilled water and was used for the preparation of plasma standards. These calibration standards were made in drug-free human plasma to yield concentrations of 100, 250, 500 and 1000 ng/ml of amisulpride. In the same manner, plasma quality controls (Q.C.) spiked with 150, 350 and 750 ng/ml of amisulpride were prepared to evaluate accuracy and precision.

2.4. Sample preparation

One hundred μl of I.S. (30 μg/ml) and 100 μl of 2 N NaOH were added to 1 ml of calibration or patients plasma. The mixture was extracted with 7 ml of hexane–isoamylic alcohol (98:2, v/v) by rotative shaking during 20 min. After centrifugation, the organic phase was added to 200 μl of 0.05 N HCl. The mixture was shaken during 15 min and centrifuged. The upper organic phase was discarded and 40 μl of aqueous phase was injected into the chromatographic apparatus.

2.5. Accuracy, precision and recovery

The accuracy and intra- and inter-day precision of the method were evaluated by assaying replicates of Q.C. samples prepared as described above. The intra-day precision was defined by calculating the coefficients of variation (C.V.) for Q.C. samples with eight replicates. The inter-day precision was determined from Q.C. samples obtained on eight different days. Accuracy, expressed as bias, was calculated as the difference (in %) between the amount of amisulpride added and recovered.

Extraction recoveries from human plasma were determined by comparison of HPLC responses from extracted samples, containing low (100 ng/ml) and high (1000 ng/ml) concentrations of amisulpride, to

those from unextracted and directly injected standards containing the same amounts.

3. Results and discussion

The objective of this work was the development of a specific, rapid and easy RP-HPLC assay with a total run time <5 min while maintaining suitable sensitivity and selectivity.

Under the described RP-HPLC procedure, amisulpride and viloxazine (I.S.) were sufficiently resolved from endogenous plasma compounds and their retention times were approximately 3 min for amisulpride and 4 min for I.S., respectively. Representative chromatograms of plasma samples are illustrated in Fig. 1.

3.1. Precision, accuracy and linearity

The results obtained for precision and accuracy are listed in Table 1 and expressed as C.V. (%) and percent bias, respectively. These results indicate that the method is precise: intra-day precision was within 3.6% and inter-day precision was within 4.9% of the relative standard deviation. This method is accurate (bias ranged from 0.01 to 5.0%).

Table 1
Precision and accuracy of results for plasma spiked with amisulpride (n=8)

	Concentrations added (ng/ml)	Concentrations found±SD (ng/ml)	C.V. (%)	Bias (%)
Intra-day	150	155.4±3.6	2.3	−3.6
	350	354.6±6.7	1.9	−1.3
	750	758.9±27.5	3.6	−1.2
Inter-day	150	157.6±7.8	4.9	−5.0
	350	354.9±16.3	4.6	−1.4
	750	750.6±30.8	4.1	−0.01

From eight calibration curves, constructed with calibration points ranging from 100 to 1000 ng/ml, a high correlation coefficient (r) was found: 0.985 (P<0.0001). This linear correlation had a slope of

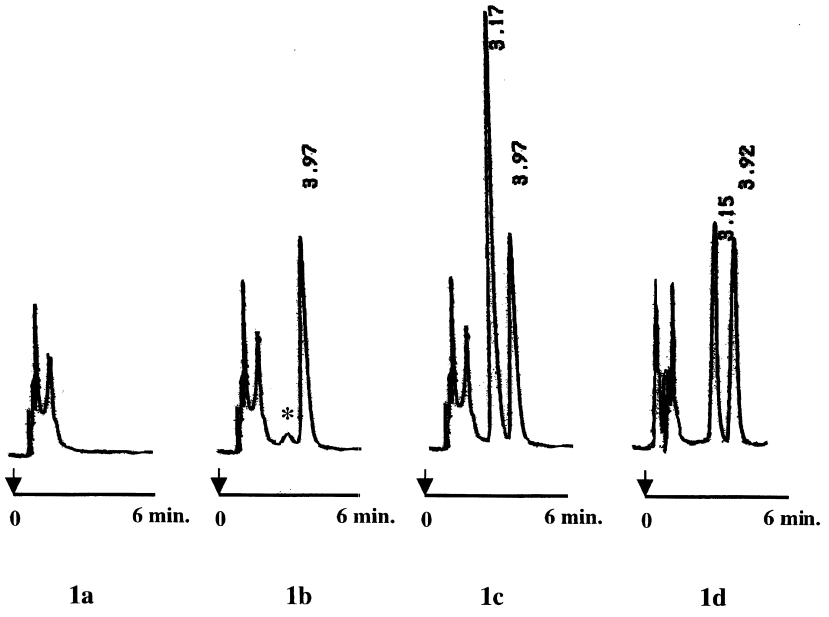

Fig. 1. Chromatograms of (a) blank plasma sample; (b) spiked plasma (at LOQ=20 ng/ml*); (c) spiked plasma (1000 ng/ml) and (d) plasma extract of patient 2 (564 ng/ml). Amisulpride: t_r=3.1 min; internal standard: t_r=3.9 min.

0.0018 (±0.0005) and an intercept of 0.0054 (±0.029).

3.2. Limit of quantification (LOQ)

The LOQ was defined as the lowest amisulpride concentration that could be determined with a precision less than 20% (C.V.) and with an accuracy between ±20% (bias), as determined in the inter-day analytical runs. The LOQ was found to be 20 ng/ml (Fig. 1).

3.3. Extraction efficiency

The mean recovery of amisulpride obtained from two different concentrations (100 and 1000 ng/ml) was 98.9±0.8%. Whatever the concentration level, the recovery was good.

3.4. Stability

To determine the influence of temperature on the stability of the drug, two Q.C. samples spiked with amisulpride were stored under different conditions: at −20 °C during 1 month; at +4 °C during 48 h. Two groups of samples were stored at +20 °C during 24 h in the daylight, and in the dark, respectively.

No tendency for decomposition was noticed in the quick frozen samples during 1 month. Indeed, at −20 °C, the percent variation coefficient (C.V.) was equal or inferior to 4.7% and the accuracy presented a bias percent inferior to 5%. The storage during 48 h at +4 °C produced no significant decrease of amisulpride concentration (C.V. and percent bias values were less than 6%). Finally, the storage at room temperature during 24 h in the daylight or in the dark indicated a good stability in the two cases with C.V. equal or inferior to 6.6% and percent bias values less than 9%.

3.5. Clinical cases

In case of acute intoxications, the ingested dose and the beginning of intoxication were often unknown. Nevertheless, the expected concentrations are generally higher than the therapeutic range. By using the described method, we were able to manage eight cases of amisulpride poisoning. In our study (Table 2), five patients were above the concentrations of amisulpride generally found at therapeutic dosages. The therapeutic plasma concentrations are less than 200 ng/ml [2].

Table 2
Concentrations of amisulpride in eight acute intoxications

Patient no.	Amisulpride (ng/ml)
1	107
2	564
3	8135[a]
4	1200[a]
5	121
6	14 824[a]
7	5602[a]
8	<20

[a] For quantification, appropriate dilutions of these plasma samples were made in drug-free human plasma.

According to the literature [3], amisulpride is generally well tolerated at the therapeutic dosages (400–1200 mg/day). But in case of poisoning, this substituted benzamide neuroleptic may induce seizures and cardiac toxicity. Traqui et al. [10] observed tachycardia and slight prolongation of the QT interval with blood concentration of amisulpride around 10 μg/ml. In our study, patient 6 presented concentrations 100-fold above the therapeutic concentration after ingestion of 8 g of amisulpride. He showed symptoms of cardiac toxicity with a QT prolongation.

Finally, the reported HPLC method is specific and easy to perform, allowing rapid determination of plasma amisulpride concentrations. The limit of quantification and short duration of this assay are particularly adapted to the management of acute amisulpride intoxications.

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